

Code No. 27187

Rat COX-2 Assay Kit - IBL

INTRODUCTION

Cyclooxygenase (COX) is a membrane bound enzyme responsible for the oxidation of arachidonic acid to Prostaglandin G₂ (PGG₂) and the subsequent reduction of PGG₂ to PHG₂. These reactions are the first steps in the formation of a variety of prostanoids. COX has been shown to be expressed in at least two different isoforms, a constitutively expressed form, COX-1, and an inducible form, COX-2. COX-1 is thought to regulate a number of housekeeping functions, such as vascular hemostasis, renal blood flow, and maintenance of glomerular function. Inflammation mediators such as growth factors, cytokines and endotoxin induce COX-2 expression in a number of cellular systems.

PRINCIPLE

This kit is a solid phase sandwich ELISA using 2 kinds of high specific antibodies. Tetra Methyl Benzidine (TMB) is used as a coloring agent (Chromogen). The strength of coloring is proportional to the quantities of Rat COX-2.

MEASUREMENT RANGE

0.36 - 23 ng/mL

INTENDED USE

- The IBL's Rat COX-2 Kit is capable of the quantitative determination of Rat COX-2 in lysate of cells or tissue. (Protocol example)
For cultured cells
 - 1) Put cultured cells (1x10⁵-1x10⁷ cells) in 0.5 mL of IBLysis-I (product code #19022).
 - 2) Mix thoroughly on a Vortex or by pipetting.
 - 3) Solubilize by rotation at 2-8°C for 30 minutes.
 - 4) Centrifuge at 10,000 rpm for 10 minutes at 2-8°C.
 - 5) Apply the supernatant diluted by "4, EIA buffer" as necessary.
- For tissue
 - 1) Put an appropriate amount of tissue to IBLysis-I.
 - 2) Homogenize with a homogenizer on ice.
 - 3) Solubilize by leaving it undisturbed for 4 hours at 2-8°C
 - 4) Centrifuge at 10,000 rpm for 10 minutes at 2-8°C.
 - 5) Apply the supernatant diluted by "4, EIA buffer" as necessary.
- In the assay for cell lysate, it is recommend that the same number of cells is used.
- It is recommend that total protein concentration is assayed at the same time in order to determine the proportion of Rat COX-2 to total protein.

KIT COMPONENT

1	Precoated plate: Anti- COX-2 (C) Rabbit IgG Affinity Purify	96Well x 1
2	Labeled antibody Conc. : (30X) HRP conjugated Anti- Rat COX-2 (C251) Rabbit IgG Fab' Affinity Purify	0.4mL x 1
3	Standard : Recombinant Rat COX-2	0.5mL x 2
4	EIA buffer*	30mL x 1
5	Solution for Labeled antibody*	12mL x 1
6	Chromogen : TMB solution	15mL x 1
7	Stop solution*	12mL x 1
8	Wash buffer Conc.*	50mL x 1

OPERATION MANUAL

1. Materials needed but not supplied

- Plate reader (450nm)
- Graduated cylinder and beaker
- Refrigerator (as 4°C)
- Paper towel
- Plate washer or washing bottle*
- Disposable test tube for "2, Labeled antibody Conc." and "6, Chromogen"
- Micropipette and tip
- Deionized water
- Graph paper (log/log)
- Tube for dilution of Standard

2. Preparation

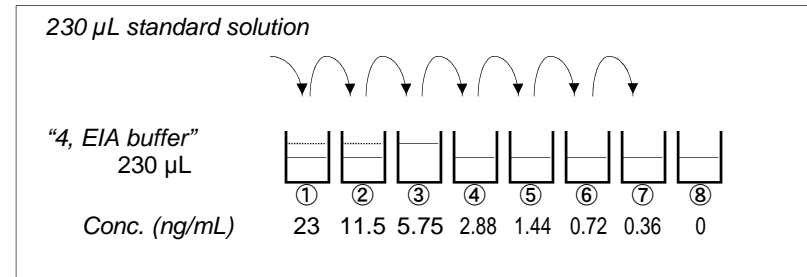
- 1) Preparation of wash buffer
"8, Wash buffer Conc." is a concentrated (40X) buffer. Adjust the temperature of "8, Washing buffer Conc." to room temperature and then, mix it gently and completely before use. Dilute 50 mL of "8, Wash buffer Conc." with 1,950 mL of deionized water and mix it. This is the wash buffer for use. This prepared wash buffer shall be stored in refrigerator and used within 2 weeks after dilution.
- 2) Preparation of Labeled antibody
"2, Labeled antibody Conc." is a concentrated (30X). Dilute "2, Labeled antibody Conc." with "5, Solution for Labeled antibody" in 30 times according to required quantity into a disposable test tube. Use this resulting solution as Labeled antibody.
Example)
In case you use one strip (8 well), the required quantity of Labeled antibody is 800 µL. (Dilute 30 µL of "2, Labeled antibody Conc." with 870 µL of "5, Solution for Labeled antibody" and mix it. And use the resulting solution by 100 µL in each well.)
This operation should be done just before the application of Labeled antibody.
The remaining "2, Labeled antibody Conc." should be stored at 4°C in firmly sealed vial.
- 3) Preparation of Standard
Put just 0.5 mL of "4, EIA buffer" into the vial of "3, Standard" and mix it gently and completely. This solution is 46 ng/mL Rat COX-2 standard.
- 4) Dilution of Standard
Prepare 8 tubes for dilution of "3, Standard". Put 230 µL each of "4, EIA buffer" into the tube.
Specify the following concentration of each tube."

Tube-1	23 ng/mL
Tube-2	11.5 ng/mL
Tube-3	5.75 ng/mL
Tube-4	2.88 ng/mL
Tube-5	1.44 ng/mL
Tube-6	0.72 ng/mL
Tube-7	0.36 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Tube-2	11.5 ng/mL
Tube-3	5.75 ng/mL
Tube-4	2.88 ng/mL
Tube-5	1.44 ng/mL
Tube-6	0.72 ng/mL
Tube-7	0.36 ng/mL
Tube-8	0 ng/mL (Test Sample Blank)

Put 230 µL of Standard solution into tube-1 and mix it gently. Then, put 230 µL of tube-1 mixture into tube-2. Dilute two times standard solution in series to set up 7 points of diluted standard between 23 ng/mL and 0.36 ng/mL. Tube-8 is the test sample blank as 0 ng/mL.

See following picture.



5) Dilution of test sample

Test sample should be diluted with "4, EIA buffer" as necessary. If the concentration of Rat COX-2 in samples may not be estimated in advance, the pre-assay with several different dilutions will be recommended to determine the proper dilution of samples.

3. Measurement procedure

All reagents shall be brought to room temperature approximately 30 minutes before use. Then mix it gently and completely before use. Make sure of no change in quality of the reagents. Standard curve shall be prepared simultaneously with the measurement of test samples.

	Test Sample	Standard	Test Sample Blank	Reagent Blank
Reagents	Test sample 100 µL	Diluted standard (Tube 1-7) 100 µL	EIA buffer (Tube-8) 100 µL	EIA buffer 100 µL
Incubation overnight at 4°C with plate lid				
4 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)*				
Labeled Antibody	100 µL	100 µL	100 µL	-
Incubation for 30 minutes at 4°C with plate lid				
5 times (wash buffer more than 350 µL) (Refer to No. 8 and 9 described in OPERATING PRECAUTION.)*				
Chromogen	100 µL	100 µL	100 µL	100 µL
Incubation for 30 minutes at room temperature (shielded)				
Stop solution	100 µL	100 µL	100 µL	100 µL
Read the plate at 450nm against a Reagent Blank within 30 minutes after addition of Stop solution.				

- 1) Determine wells for reagent blank. Put 100 µL each of "4, EIA buffer" into the wells.
- 2) Determine wells for test sample blank, test sample and diluted standard. Then, put 100 µL each of test sample blank (tube-8), test sample and dilutions of standard (tube-1-7) into the appropriate wells.
- 3) Incubate the precoated plate overnight at 4°C after covering it with plate lid.
- 4) Wash the plate with the prepared wash buffer and remove all liquid.*
- 5) Pipette 100 µL of labeled antibody solution into the wells of test samples, diluted standard and test sample blank.
- 6) Incubate the precoated plate for 30 minutes at 4°C after covering it with plate lid.
- 7) Wash the plate with the prepared wash buffer and remove all liquid.*
- 8) Take the required quantity of "6, Chromogen" into a disposable test tube. Then, pipette 100 µL from the test tube into the wells. Please do not return the rest of the test tube to "6, Chromogen" bottle to avoid contamination.
- 9) Incubate the precoated plate for 30 minutes at room temperature in the dark. The liquid will turn blue by addition of "6, Chromogen".
- 10) Pipette 100 µL of "7, Stop solution" into the wells. Mix the liquid by tapping the side of precoated plate. The liquid will turn yellow by addition of "7, Stop solution".
- 11) Remove any dirt or drop of water on the bottom of the precoated plate and confirm there is no bubble on the surface of the liquid. Then, run the plate reader and conduct measurement at 450 nm against a reagent blank. The measurement shall be done within 30 minutes after addition of "7, Stop solution".

OPERATING PRECAUTION*

- 1) Test samples should be measured soon after collection. For storage of samples, store them frozen and do not repeat freeze/thaw cycles. Thaw the test samples at a low temperature and mix them completely before measurement.
- 2) Test samples should be diluted with "4, EIA buffer" contained in this kit.
- 3) Duplicate measurement of test samples and standards is recommended.
- 4) Standard curve should run for each assay.
- 5) Use test samples in neutral pH range. The contaminations of organic solvent may affect the measurement.
- 6) All reagents should be brought to room temperature (R.T.) and mixed

completely and gently before use. After mixing them, make sure of no change in quality of the reagents.

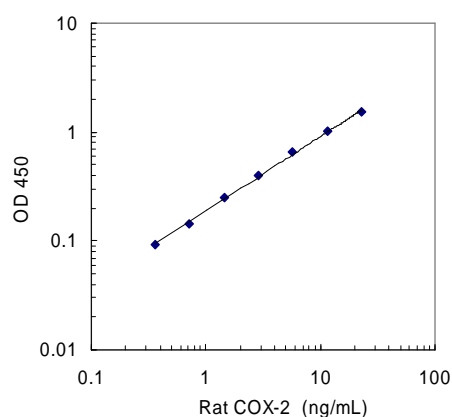
- 7) Use only "8, Wash buffer conc." contained in this kit for washing the precoated plate. Insufficient washing may lead to the failure in measurement.
- 8) Using a plate washer is recommended (wait time zero second). It should be washed by a plate washer immediately after each reaction. If you use a washing bottle instead of a plate washer, after filling wash buffer in each well, immediately turn the plate upside down and shake it off to completely remove the wash buffer. Repeat the number of times of wash defined in a table for measurement procedure described in section 3. It should be properly washed off as instructed in order to avoid any insufficient wash.
- 9) Carefully tap the plate against a clean paper towel without contacting with inside of each well to completely remove the washing buffer after repeated the determined number of wash.
- 10) "6, Chromogen - TMB solution" should be stored in the dark due to its sensitivity against light. It should be also avoided contact with metals. Required quantity should be prepared into a collecting container for each use.
- 11) After adding TMB solution into the wells, the liquid in the wells gradually changes the color in blue. In this process the plate should be in dark. Remained TMB solution in the collecting container should not be returned into the original bottle of TMB solution to avoid contamination.
- 12) Measurement of O.D. should be done within 30 minutes after addition of "7, Stop solution".

CALCULATION OF TEST RESULT

Subtract the absorbance of test sample blank from all data, including standards and unknown samples before plotting. Plot the subtracted absorbance of the standards against the standard concentration on log-log graph paper. Draw the best smooth curve through these points to construct the standard curve. Read the concentration for unknown samples from the standard curve.

Example of standard curve

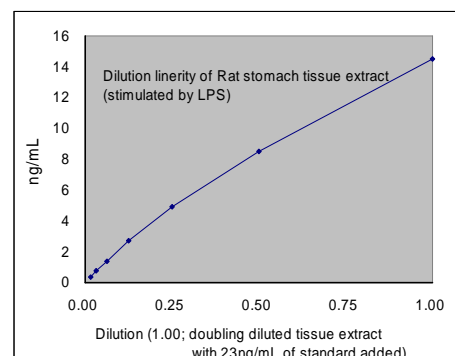
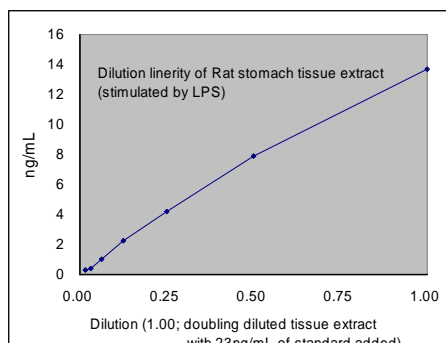
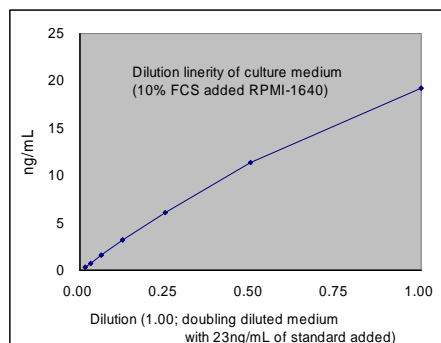
Conc. (ng/mL)	Absorbance (450nm)
23	1.618
11.5	1.101
5.75	0.747
2.88	0.497
1.44	0.343
0.72	0.241
0.36	0.189
0 (Test Sample Blank)	0.096



* The typical standard curve is shown above. This curve can not be used to derive test results. Please run a standard curve for each assay.

PERFORMANCE CHARACTERISTICS

1. Dilution Linearity (Samples with standard added are used.)



2. Added Recovery Assay

Specimen	Theoretical Value (ng/mL)	Measurement Value (ng/mL)	%
10%FCS added RPMI-1640 (x4)	11.50	11.04	96.0
	5.75	5.56	96.7
	2.88	2.73	94.8
Rat spleen extract (LPS stimulated) (x4)	12.33	8.52	69.1
	6.58	4.54	69.0
	3.71	2.80	75.5
Rat stomach extract (LPS stimulated) (x4)	11.52	7.13	61.9
	5.77	3.38	58.6
	2.90	1.54	53.1

3. Intra - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
18.41	1.02	5.5	24
4.90	0.18	3.7	24
0.96	0.06	6.3	24

4. Inter - Assay

Measurement Value (ng/mL)	SD value	CV value (%)	n
17.47	1.17	6.7	11
5.11	0.30	5.9	11
1.01	0.08	7.4	11

5. Sensitivity

0.16 ng/mL
(Calculated by NCCLS method using the standard.) *

PRECAUTION FOR INTENDED USE AND/OR HANDLING

1. All reagents should be stored at 2 - 8°C. All reagents shall be brought to room temperature approximately 30 minutes before use.
2. "3, Standard" is lyophilized products. Be careful to open this vial.
3. "7, Stop solution" is a strong acid substance. Therefore, be careful not to have your skin and clothes contact "7, Stop solution" and pay attention to the disposal of "7, Stop solution".
4. Dispose used materials after rinsing them with large quantity of water.
5. Precipitation may occur in "2, Labeled antibody Conc.", however, there is no problem in the performance.
6. Wash hands after handling reagents.
7. Do not mix the reagents with the reagents from a different lot or kit.
8. Do not use expired reagents.
9. This kit is for research purpose only. Do not use for clinical diagnosis.

STORAGE AND THE TERM OF VALIDITY

Storage Condition : 2 - 8°C
The expiry date is specified on outer box.

Version 2.

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